

We claim:

1. Substantially purified DNA comprising DNA encoding an amino acid sequence selected from the group consisting of the amino acid sequence of: (i) Streptococcus pyogenes DNase B enzyme as shown in Figure 4; and (ii) a sequence encoding a functional equivalent of S. pyogenes DNase B enzyme, the DNA being substantially free of DNA that does not encode the amino acid sequence of Figure 4 or a functional equivalent of S. pyogenes DNase B enzyme except for a leader peptide fused to the amino terminus of S. pyogenes DNase B enzyme.

2. The DNA of claim 1 wherein the DNA further comprises a DNA sequence encoding a leader peptide fused to the amino terminus of S. pyogenes DNase B enzyme.

3. The DNA of claim 1 having the nucleotide sequence of Figure 3.

4. An expression vector for Streptococcus pyogenes DNase B enzyme comprising the DNA sequence of claim 1 operatively linked to at least one control sequence compatible with a suitable bacterial host cell.

5. An expression vector for Streptococcus pyogenes DNase B enzyme comprising the DNA sequence of claim 3 operatively linked to at least one control sequence compatible with a suitable bacterial host cell.

6. The vector of claim 4 wherein the DNA encoding the Streptococcus pyogenes DNase B enzyme is linked to at least one sequence from bacteriophage  $\lambda$ .

7. The vector of claim 5 wherein the DNA encoding the Streptococcus pyogenes DNase B enzyme is linked to at least one sequence from bacteriophage  $\lambda$ .

5 8. A bacterial host cell transformed with the expression vector of claim 4 in a manner allowing the transformed bacterial host cell to express the Streptococcus pyogenes DNase B encoded by the DNA incorporated within the expression vector of claim 4 in a detectable quantity.

10 9. A bacterial host cell transformed with the expression vector of claim 5 in a manner allowing the transformed bacterial host cell to express the Streptococcus pyogenes DNase B encoded by the DNA incorporated within the expression vector of claim 5 in a detectable quantity.

15 10. Substantially purified S. pyogenes DNase B enzyme comprising a protein ~~having~~ the amino acid sequence of Figure 4.

20 11. A process for producing substantially purified Streptococcus pyogenes DNase B enzyme comprising:

- 25 (a) culturing the bacterial host cell of claim 8;  
(b) using the cultured bacterial host cell to express the DNase B enzyme; and  
(c) purifying the enzyme from the cultured bacterial host cell.

30 12. A process for producing substantially purified Streptococcus pyogenes DNase B enzyme comprising:

- (a) culturing the bacterial host cell of claim 9;  
(b) using the cultured bacterial host cell to express the DNase B enzyme; and  
(c) purifying the enzyme from the cultured bacterial host cell.

13. Streptococcus pyogenes DNase B enzyme prepared by the process of claim 11.

14. Streptococcus pyogenes DNase B enzyme prepared by the process of claim 12.

15. Streptococcus pyogenes DNase B enzyme fused at its amino terminus with a leader peptide, the leader peptide having the sequence M-N-L-L-G-S-R-R-V-F-S-K-K-C-R-L-V-K-F-S-M-V-A-L-V-S-A-T-M-A-V-T-T-V-T-L-E-N-T-A-L-A-R (SEQ ID NO: 1).

16. A mutant of the protein whose amino acid sequence is shown in Figure 4 in which at least one of the following mutations occurs:

(a) a deletion of one or more amino acids from the sequence of Figure 4;

(b) an insertion of one or more naturally-occurring L-amino acids into the sequence of Figure 4; and

(c) replacement of at least one of the amino acids of Figure 4 with an alternative naturally occurring L-amino acid;

the resulting mutant having reduced or increased DNase B activity or another altered property.

17. The mutant protein of claim 16 wherein the mutant substantially maintains the antigenic reactivity of natural S. pyogenes DNase B enzyme.

18. A transcriptional fusion comprising at least a portion of the S. pyogenes DNase B DNA sequence of claim 3 fused with another gene, with the fusion having a detectable property altered from the property of the sequence of claim 3.

19. A translational fusion comprising at least a portion of the protein coded for by the S. pyogenes DNase B protein sequence of claim 3 fused with another protein, with

the fusion having a detectable property altered from the property of the protein coded for by the sequence of claim 3.

5           20. Substantially purified Streptococcus pyogenes DNase B enzyme substantially free of proteins other than: (1) the Streptococcus DNase B enzyme and (2) Streptococcus DNase B enzyme fused at its amino terminus with a leader peptide, the substantially purified protein being substantially free of  
10 mitogenic activity.

21. The substantially purified S. pyogenes DNase B enzyme of claim 20 comprising Fraction I of S. pyogenes DNase B enzyme and substantially free of Fraction II of S. pyogenes  
15 DNase B enzyme.

22. The substantially purified S. pyogenes DNase B enzyme of claim 20 comprising Fraction II of S. pyogenes DNase B enzyme and substantially free of Fraction I of S. pyogenes  
20 DNase B enzyme.

23. A process for preparing substantially purified Streptococcus pyogenes DNase B enzyme comprising:

- 25           (a) absorption to and elution from diethylaminoethyl cellulose to produce a first eluate;  
            (b) chromatography of the first eluate on phenyl agarose to produce a second eluate;  
            (c) chromatography of the second eluate on heparin agarose to produce a third eluate; and  
30           (d) chromatofocusing of the third eluate to produce substantially purified DNase B enzyme.

24. The process of claim 23 further comprising purification of the substantially purified DNase B by reverse-phase high-pressure liquid chromatography.  
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25. Substantially ~~purified~~ Streptococcus pyogenes DNase B enzyme produced by the process of claim 23.

26. A single-stranded nucleic acid probe hybridizing with the DNA sequence coding for the amino-terminal 23 amino acids of the Streptococcus pyogenes DNase B enzyme, not including any portion of the leader sequence thereof, with no greater than about a 30% mismatch.

27. An antibody specifically binding the Streptococcus pyogenes DNase B enzyme of claim 13.

28. An antibody specifically binding the Streptococcus pyogenes DNase B enzyme of claim 14.

29. An antibody specifically binding the Streptococcus pyogenes DNase B enzyme of claim 20.

30. An antibody specifically binding the Streptococcus pyogenes DNase B enzyme of claim 25.

31. A monoclonal antibody specifically binding the Streptococcus pyogenes DNase B enzyme of claim 13.

32. A monoclonal antibody specifically binding the Streptococcus pyogenes DNase B enzyme of claim 14.

33. A monoclonal antibody specifically binding the Streptococcus pyogenes DNase B enzyme of claim 20.

34. A monoclonal antibody specifically binding the Streptococcus pyogenes DNase B enzyme of claim 25.

35. A method for detecting and/or determining anti-Streptococcus pyogenes DNase B antibody in a test sample, comprising the steps of:

(a) providing a test sample suspected of containing anti-Streptococcus pyogenes DNase B antibody;

(b) adding a quantity of the Streptococcus pyogenes DNase B enzyme of claim 13 to the test sample, the quantity being sufficient to produce a detectable level of enzymatic activity in the absence of inhibition of the enzymatic activity by anti-DNase B antibody in the test sample; and

(c) determining a level of activity of DNase B enzyme in the test sample by performing an enzyme assay to detect and/or determine the anti-Streptococcus pyogenes antibody in the test sample.

36. A method for detecting and/or determining anti-Streptococcus pyogenes DNase B antibody in a test sample, comprising the steps of:

(a) providing a test sample suspected of containing anti-Streptococcus pyogenes DNase B antibody;

(b) adding a quantity of the Streptococcus pyogenes DNase B enzyme of claim 14 to the test sample, the quantity being sufficient to produce a detectable level of enzymatic activity in the absence of inhibition of the enzymatic activity by anti-DNase B antibody in the test sample; and

(c) determining the level of antibody of DNase B enzyme in the test sample to detect and/or determine the anti-Streptococcus pyogenes antibody in the test sample.

37. A method for detecting and/or determining anti-Streptococcus pyogenes DNase B antibody in a test sample, comprising the steps of:

(a) providing a test sample suspected of containing anti-Streptococcus pyogenes DNase B antibody;

(b) adding a quantity of the Streptococcus pyogenes DNase B enzyme of claim 20 to the test sample, the quantity being sufficient to produce a detectable level of enzymatic activity in the absence of inhibition of the enzymatic activity by anti-DNase B antibody in the test sample, and

(c) determining the level of activity of DNase B enzyme in the test sample by performing an enzyme assay to detect and/or determine anti-Streptococcus pyogenes antibody in the test sample.

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38. A method for detecting and/or determining anti-Streptococcus pyogenes DNase B antibody in a test sample, comprising the steps of:

10 (a) providing a test sample suspected of containing anti-Streptococcus pyogenes DNase B antibody;

(b) adding a quantity of the Streptococcus pyogenes DNase B enzyme of claim 25 to the test sample, the quantity being sufficient to produce a detectable level of enzymatic activity in the absence of inhibition of the enzymatic  
15 activity by anti-DNase B antibody in the test sample; and

(c) determining the level of activity of DNase B enzyme in the test sample by performing an enzyme assay to detect and/or determine anti-Streptococcus pyogenes antibody in the test sample.

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39. A method for detecting and/or determining anti-Streptococcus pyogenes DNase B antibody in a test sample, comprising the steps of:

25 (a) binding the Streptococcus pyogenes DNase B enzyme of claim 13 to a solid support;

(b) reacting a test sample suspected of containing anti-Streptococcus pyogenes DNase B antibody with the Streptococcus pyogenes DNase B enzyme bound to the solid support to bind the antibody to the enzyme and thus to the  
30 solid support; and

(c) detecting the antibody bound to the solid support to detect and/or determine the antibody in the test sample.

40. A method for detecting and/or determining anti-Streptococcus pyogenes DNase B antibody in a test sample, comprising the steps of:

(a) binding the Streptococcus pyogenes DNase B enzyme of claim 14 to a solid support;

(b) reacting a test sample suspected of containing anti-Streptococcus pyogenes DNase B antibody with the Streptococcus pyogenes DNase B enzyme bound to the solid support to bind the antibody to the enzyme and thus to the solid support; and

(c) detecting the antibody bound to the solid support to detect and/or determine the antibody in the test sample.

41. A method for detecting and/or determining anti-Streptococcus pyogenes DNase B antibody in a test sample, comprising the steps of:

(a) binding the Streptococcus pyogenes DNase B enzyme of claim 20 to a solid support;

(b) reacting a test sample suspected of containing anti-Streptococcus pyogenes DNase B antibody with the Streptococcus pyogenes DNase B enzyme bound to the solid support to bind the antibody to the enzyme and thus to the solid support; and

(c) detecting the antibody bound to the solid support to detect and/or determine the antibody in the test sample.

42. A method for detecting and/or determining anti-Streptococcus pyogenes DNase B antibody in a test sample, comprising the steps of:

(a) binding the Streptococcus pyogenes DNase B enzyme of claim 25 to a solid support;

(b) reacting a test sample suspected of containing anti-Streptococcus pyogenes DNase B antibody with the Streptococcus pyogenes DNase B enzyme bound to the solid

support to bind the antibody to the enzyme and thus to the solid support; and

(c) detecting the antibody bound to the solid support to detect and/or determine the antibody in the test sample.

43. A method for detecting and/or determining anti-Streptococcus pyogenes DNase B antibody in a test sample, comprising the steps of:

(a) preparing a buffered solution of the DNase B of claim 13;

(b) reacting the buffered DNase B solution with a test sample suspected of containing anti-S. pyogenes DNase B antibody; and

(c) detecting a reaction between the DNase B and the anti-DNase B antibody by observing and/or measuring a change in light absorption and/or light scattering in the solution.

44. A method for detecting and/or determining anti-Streptococcus pyogenes DNase B antibody in a test sample, comprising the steps of:

(a) preparing a buffered solution of the DNase B of claim 14;

(b) reacting the buffered DNase B solution with a test sample suspected of containing anti-S. pyogenes DNase B antibody; and

(c) detecting a reaction between the DNase B and the anti-DNase B antibody by observing and/or measuring a change in light absorption and/or light scattering in the solution.

45. A method for detecting and/or determining anti-Streptococcus pyogenes DNase B antibody in a test sample, comprising the steps of:

(a) preparing a buffered solution of the DNase B of claim 20;

(b) reacting the buffered DNase B solution with a test sample suspected of containing anti-S. pyogenes DNase B antibody; and

(c) detecting a reaction between the DNase B and the anti-DNase B antibody by observing and/or measuring a change in light absorption and/or light scattering in the solution.

46. A method for detecting and/or determining anti-Streptococcus pyogenes DNase B antibody in a test sample, comprising the steps of:

(a) preparing a buffered solution of the DNase B of claim 25;

(b) reacting the buffered DNase B solution with a test sample suspected of containing anti-S. pyogenes DNase B antibody; and

(c) detecting a reaction between the DNase B and the anti-DNase B antibody by observing and/or measuring a change in light absorption and/or light scattering in the solution.

47. A method of using a promoter originally associated with the S. pyogenes DNase B gene to express a protein other than DNase B comprising:

(a) separating the promoter originally associated with the S. pyogenes DNase B gene from the S. pyogenes DNase B gene;

(b) operatively linking the promoter with a structural gene for a S. pyogenes protein other than the gene for DNase B; and

(c) expressing the protein encoded by the structural gene.

48. The method of claim 47 wherein the protein is expressed in S. pyogenes.

49. The method of claim 48 wherein the protein is expressed in a prokaryote other than S. pyogenes.

50. A substantially purified promoter sequence derived from a promoter sequence originally associated with S. pyogenes DNase B including therein a start site for transcription and sites homologous to the consensus -10 and -35 sites of bacterial promoters.

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51. A process for using a leader peptide associated with S. pyogenes DNase B enzyme to express a protein in a prokaryote comprises:

10 (1) fusing the DNA coding for the protein to DNA coding for a leader peptide, the leader peptide having the sequence M-N-L-L-G-S-R-R-V-F-S-K-K-C-R-L-V-K-F-S-M-V-A-L-V-S-A-T-M-A-V-T-T-V-T-L-E-N-T-A-L-A-R (SEQ ID NO: 1), so that the fused DNA codes for a recombinant protein with a single  
15 reading frame with the leader peptide being at the amino-terminus of the protein;

(2) introducing the fused DNA into the prokaryote;  
and

20 (3) expressing the fused DNA in the prokaryote so that the recombinant protein is produced in a recoverable quantity.

52. The process of claim 51 wherein the prokaryote is E. coli.

25 53. The process of claim 51 wherein the prokaryote is a gram-positive bacterium selected from Staphylococcus, Streptococcus, and Streptomyces species.

30 54. The process of claim 51 wherein the recombinant protein is excreted into the culture medium of the prokaryote.

35 55. A method for immunizing a mammal against infection with S. pyogenes comprising administering a quantity of the purified S. pyogenes DNase B enzyme of claim 13 to the

mammal sufficient to stimulate production of antibodies specific for S. pyogenes DNase B.

56. A method for immunizing a mammal against infection with S. pyogenes comprising administering a quantity of the purified S. pyogenes DNase B enzyme of claim 14 to the mammal sufficient to stimulate production of antibodies specific for S. pyogenes DNase B.

57. A method for immunizing a mammal against infection with S. pyogenes comprising administering a quantity of the purified S. pyogenes mutant DNase B enzyme of claim 17 to the mammal sufficient to stimulate production of antibodies specific for S. pyogenes DNase B.

58. A method for immunizing a mammal against infection with S. pyogenes comprising administering a quantity of the purified S. pyogenes DNase B enzyme of claim 20 to the mammal sufficient to stimulate production of antibodies specific for S. pyogenes DNase B.

59. A method for immunizing a mammal against infection with S. pyogenes comprising administering a quantity of the purified S. pyogenes DNase B enzyme of claim 25 to the mammal sufficient to stimulate production of antibodies specific for S. pyogenes DNase B.

60. A method for treating cystic fibrosis in a patient with cystic fibrosis comprising:

- (a) generating an aerosol of the purified enzymatically active DNase B enzyme of claim 13; and
- (b) administering the aerosol to a patient with cystic fibrosis in a quantity sufficient to reduce lung fluid viscosity in the patient.

61. A method for treating cystic fibrosis in a patient with cystic fibrosis comprising:

(a) generating an aerosol of the purified enzymatically active DNase B enzyme of claim 14; and

(b) administering the aerosol to a patient with cystic fibrosis in a quantity sufficient to reduce lung fluid viscosity in the patient.

62. A method for treating cystic fibrosis in a patient with cystic fibrosis comprising:

(a) generating an aerosol of the purified enzymatically active DNase B enzyme of claim 20; and

(b) administering the aerosol to a patient with cystic fibrosis in a quantity sufficient to reduce lung fluid viscosity in the patient.

63. A method for treating cystic fibrosis in a patient with cystic fibrosis comprising:

(a) generating an aerosol of the purified enzymatically active DNase B enzyme of claim 25; and

(b) administering the aerosol to a patient with cystic fibrosis in a quantity sufficient to reduce lung fluid viscosity in the patient.